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学 位 論 文 要 旨

ABSTRACT

Transgenic mice carrying the coding sequence of the ß-galactosidase gene whose expression is driven by various upstream regions, including the transcription promoter, of the testis-specific mouse *Pgk-2* gene, were generated. Expression of ß-galactosidase mRNA from the transgene containing the region between nucleotide positions –1404 and +61 with respect to the transcription initiation site numbered +1, was examined by reverse transcription-mediated polymerase chain reaction, Northern blotting, dot blot hybridization, and *in situ* hybridization, and compared with expression of the endogenous Pgk-2 mRNA. These results revealed that the 1.4-kbp DNA region is sufficient for determining the tissue-, developmental stage- and spermatogenic stage-specific transcription of the mouse *Pgk-2* gene. When the region between –684 and +61 was used to generate transgenic mice, ß-galactosidase mRNA was detectable not only in the testis but in other tissues such as brain and lung. However, the timing and cell-type specificity of ß-galactosidase mRNA expression in the testis were retained in these mice. These results indicated that the region between –684 and +61 is sufficient for testicular control of the *Pgk-2* gene expression, but another DNA region located far upstream is needed to restrict its expression to the testis

Introduction

The mammalian spermatogenic pathway consists of complex series of events, including proliferation and differentiation of spermatogonial stem cells, meiotic cell division of differentiating spermatogenic cells, and morphogenic maturation of spermatids. The expression of a number of genes is altered during this process, and the resulting repertoire of testis-specific proteins is seemingly required both for advancing the spermatogenic pathway and for maintaining the architectural organization of spermatozoa. It is thus important to elucidate the mechanism for regulation of spermatogenic gene expression in order to understand the molecular basis of mammalian spermatogenesis.

There are two isozymes for the glycolytic enzyme phosphoglycerate kinase (Pgk), somatic-type Pgk-1 and testis-specific Pgk-2, which are coded for by distinct genes. In the testis, Pgk-1 mRNA is present in both somatic and pre-meiotic spermatogenic cells. As spermatogenesis proceeds, the amount of Pgk-1 mRNA decreases and Pgk-2 mRNA, on the other hand, becomes detectable first in spermatocytes. It is thus likely that *Pgk-2* gene transcription is activated in differentiating spermatogenic cells to compensate for the loss of Pgk-1.

The mechanism underlying the transcription switch from *Pgk-1* to *Pgk-2* has been studied by *in vitro* experiments. The previous results suggested that spermatogenic expression of the mouse *Pgk-2* gene is regulated by both positive and negative *cis*-acting DNA elements. The positive regulatory sequence is located in the region between nucleotide positions –64 and –82 with respect to the transcription initiation site numbered +1, and an Ets-like nuclear protein(s) binds to it. The negative *cis*-element functions as a silencer and consists of two separate DNA regions. In the present study, I examined the role of the upstream region in spermatogenic transcription of the mouse *Pgk-2* gene using transgenic mice.

Result

Generation of transgenic mice carrying transcription control region of mouse *Pgk-2* gene

In the previous *in vitro* experiments, the region between nucleotide positions –1404 and –685 was shown to act as a silencer, which consisted of

two negative *cis*-elements, negative-D and negative-P. I therefore decided to determine the function of these two DNA regions in regulating spermatogenic expression of the *Pgk-2* gene using transgenic mice. Fertilized eggs were injected with two transgene constructs that contained the upstream regions of the *Pgk-2* gene fused to the coding sequence of ß-galactosidase (Fig. 1). DNA extracted from tails of the offspring was tested for the presence of the transgene by Southern blotting, and the presence of ß-galactosidase activity in the testis was confirmed by staining testis sections with 5-bromo-4-chloro-3-indolyl-ß-D-galactoside (X-gal), a histochemical substrate of the enzyme. I then randomly chose three of the X-gal-positive lines for further analyses.

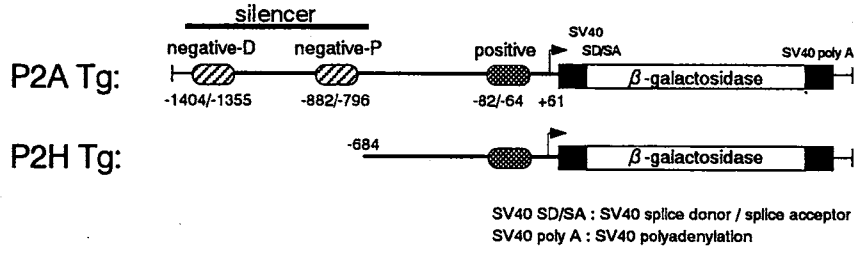

Fig. 1. Structure of P2A and P2H transgenes

Tissue- and spermatogenic cell type-specific expression of ß-galactosidase mRNA in P2A-Tg lines

I first analyzed the P2A-Tg lines. To determine the tissue specificity of ß-galactosidase mRNA expression, total RNA was extracted from various tissues and analyzed for the presence of ß-galactosidase, Pgk-2, and Pgk-1 mRNAs by RT-PCR and Northern blotting. Pgk-2 mRNA was present exclusively in the testis, while Pgk-1 mRNA was detected in all the tissues examined, and the distribution of ß-galactosidase mRNA was similar to that of Pgk-2 mRNA. All three transgenic lines tested gave essentially identical results, and there was no appreciable correlation between the number of integrated transgenes and the level of ß-galactosidase mRNA in the three lines. These results indicated that the 1.4-kbp upstream region of the mouse *Pgk-2* gene satisfactorily delimits transcription of the adjacent ß-galactosidase sequence to the testis.

In order to determine the spermatogenic cell-type specificity of ß-galactosidase mRNA expression, mRNA in the testis of P2A-Tg mice at various ages was analyzed by RT-PCR and *in situ* hybridization. When testicular RNA

prepared from P2A-Tg lines was subjected to RT-PCR analysis, Pgk-1 mRNA was present in all RNA samples whereas Pgk-2 mRNA was not detectable until the age of 15 days. The expression pattern of ß-galactosidase mRNA was the same as that of Pgk-2 mRNA. In *in situ* hybridization, signals derived from the ß-galactosidase sequence were detected in the cytoplasm of meiotic and post-meiotic spermatogenic cells, but not in that of pre-meiotic spermatogenic cells, *Sertoli cells*, or cells present in the testicular interstitium. Distribution of the ß-galactosidase signal was pretty match the same as that of Pgk-2 mRNA. These results indicated that the 1.4-kbp upstream region is also sufficient for spermatogenic cell type-specific transcription of the mouse *Pgk-2* gene.

Ectopic activation of *Pgk-2* promoter in P2H-Tg lines

When similar analyses were conducted with P2H-Tg lines, ß-galactosidase mRNA was detectable not only in the testis but in other tissues. RT-PCR experiments showed a clear signal derived from ß-galactosidase mRNA in RNA prepared from the brain. Examination of three P2H-Tg lines revealed that ß-galactosidase mRNA was also present in many tissues, such as liver, kidney, heart, lung, and spleen. The pattern of testicular expression of ß-galactosidase mRNA in P2H-Tg lines was exactly the same as that in P2A-Tg lines in terms of development and spermatogenic differentiation. Essentially the same results were obtained with all three independent lines examined. Taking all these results into consideration, it is likely that the region between nucleotide positions –1404 and –685 functions as a negative *cis*-element to repress the *Pgk-2* promoter in non-testicular tissues.

Discussion & Conclusion

The expression pattern of ß-galactosidase mRNA in P2A-Tg lines was almost the same in all three independent lines examined and closely resembled that of endogenous Pgk-2 mRNA, indicating that expression of the transgene did not depend on where it was integrated in chromosomes. We thus conclude that the 1.4-kbp region upstream of the mouse *Pgk-2* coding sequence is sufficient for tissue-, developmental stage- and spermatogenic stage-specific transcription of the cognate gene. The mammalian *Pgk-2* gene is transcribed exclusively in the testis. This led us to anticipate the existence of a regulatory

mechanism which strictly represses *Pgk-2* transcription in other tissues. In fact, the loss of the region between nucleotide positions–1404 and –685 led to ectopic activation of the *Pgk-2* promoter in the brain and other non-testicular tissues.These results indicate that the –1404/-685 region represses the *Pgk-2* promoter in at least some non-testicular tissues. The region between –1404 and –685 of the mouse *Pgk-2* gene may include a silencer, that represses the transcription in tissues other than the testis. A lot of neuron-specific genes are transcribed under the control of a *cis*-element called neuron-restrictive silencer element (NRS). This silencer, when bound by a protein called neuron-restrictive silencer factor (NRSF), represses transcription of neuron-specific genes in non-neuronal cells. NRSF is present in non-neuronal cells, including glial cells, but absent in neurons. Thus, regulated expression of NRSF most likely defines neuron-specific transcription of a set of genes. Regarding the negative *cis*-acting region of the mouse *Pgk-2* gene, similar sequences are found in the silencer elements of c-mos- and protamine 2-encoding genes, which are both expressed in the testis but not in other tissues. I thus hypothesize that a *trans*-acting factor(s) binding to the *Pgk-2* silencer also regulates transcription of other genes to repress their expression in somatic cells (Fig. 2.).

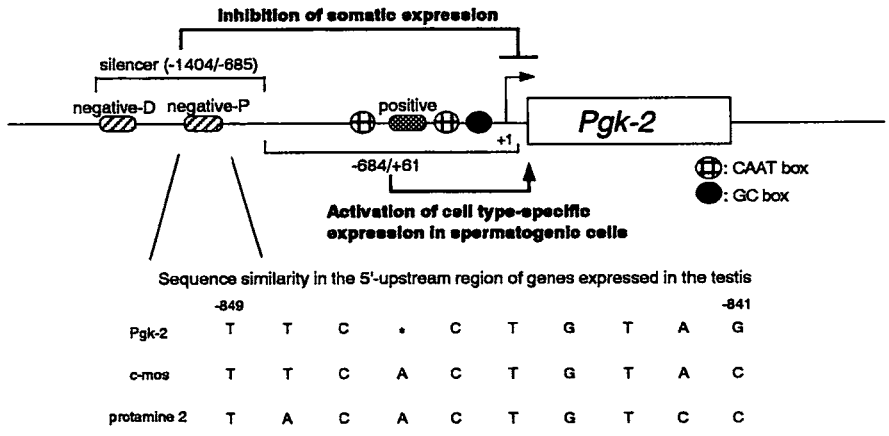

Fig. 2. A model for transcription regulation of mouse *Pgk-2* gene

学位論文審査結果の要旨

安藤博司氏から提出された学位論文について，上記５名の審査委員による査読の後に平成12年２月１日に口頭発表会が行われた。同日に最終の審査委員会が開かれ，以下の理由により当該論文は博士（薬学）の学位に値すると判定された。

本論文は，精子形成細胞特異的に発現する遺伝子の転写制御機構に関する研究結果を記述したものである。精子形成細胞で誘導される遺伝子の発現制御においては，精巣でのみ発現するという「臓器特異性」に加えて，精子形成過程の特定時期での発現という「精子分化段階特異性」が保証される必要がある。過去に行われた類似の研究には，このふたつの制御が明確に区別されて解析された例はほとんど見あたらない。

安藤氏は，トランスジェニックマウスの手法を利用して，解糖系酵素Pgkの精巣型アイソザイムPgk-2をコードする遺伝子について解析を加えた。その結果，精子形成過程特異的遺伝子転写には，転写開始点から上流684塩基対のDNA領域が必要であることが示された。また，それよりもさらに700塩基対上流までの領域に，精巣以外の臓器でこの遺伝子の転写反応を抑制する働きが担われていることが示唆された。これらの発見は，*Pgk-2*遺伝子転写の精子分化段階特異性と臓器特異性が異なる制御機構のもとにあることを示すものである。タンパク質（転写制御因子）レベルでの解析まで発展させて欲しかった感はあるが，本成果は精巣特異的遺伝子の発現制御の研究に新しい視点を与えるものと評価される。